

Allozyme diversity within and among populations of three ungulate species (*Cervus elaphus*, *Capreolus capreolus*, *Sus scrofa*) of Southeastern and Central Europe

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Abstract

75 red deer, 121 roe deer, and 155 wild boars from several sampling sites in Bulgaria were examined for genetic variability and differentiation at 40–43 isozyme loci by means of horizontal starch gel electrophoresis. For comparison with Central European populations 103 red deer, 106 roe deer and 70 wild boars from sampling sites in Slovenia, Austria, Slovakia, and France were screened additionally. Mean P (proportion of polymorphic loci, 99 per cent criterion) in the red and the roe deer was 18.6% (sd 2.3%) and 17.8% (sd 0.8%), respectively. Mean H (expected average heterozygosity) was 5.9% (sd 1.3%) in the former and 5.9% (sd 1.0%) in the latter. In both species, P- and H-values in the Bulgarian, Slovenian, and Slovakian populations were among the highest as yet detected in these species. According to genetic distances and the exclusive occurrence of rare alleles the Bulgarian red deer represents a subspecies different from Central European *Cervus elaphus hippelaphus*. The wild boars were polymorphic at 11.6% (sd 2.8%) of their loci and had a mean H of 2.8% (sd 0.5%). Genetic distances revealed some distinctness of the population living in the Rila-Rhodopi mountains from Central European *Sus scrofa scrofa*. Levels of migration (N_m) among local populations of all three species were very similar.

Introduction

The red deer (*Cervus elaphus*), the roe deer (*Capreolus capreolus*), and the wild boar (*Sus scrofa*) are among the most widely distributed European ungulate species (see TRENSE 1989). In all of them genetic diversity within and among populations has already been investigated by means of protein electrophoresis by several laboratories. Nevertheless, the studies conducted so far covered only a comparatively narrow part of the populations of each species and no attempts have been made to examine more than one species in a particular area. Considering only data which are to some extent comparable as to the number and composition of loci investigated, the red deer has been studied more extensively in southern Sweden (GYLLENSTEN et al. 1983), in Scotland (GYLLENSTEN et al. 1983; DRATCH and GYLLENSTEN 1985), in eastern France (HARTL et al. 1990a, 1991a), and in Hungary (HARTL et al. 1990a). In contrast, the roe deer has been screened almost exclusively in the Alpine area (HARTL et al. 1991b), and data on free-ranging wild boars are available only from Italy (RANDI et al. 1989, 1992) and Bulgaria (RANDI et al. 1992).

In the present study we examined genetic diversity within and among populations of red deer, roe deer, and wild boar in Bulgaria. The latter region is of particular population genetic interest, as there is considerable variation in elevation and ecological conditions (field vs. forest biotopes) within a narrow area. Furthermore, all species exhibit some morphological differences, suggesting the occurrence of two subspecies in wild boars (MARKOV 1954), a well defined field and forest ecotype in the roe deer (MARKOV et al. in prep.) and also some distinctness between red deer from the central part of the Balkan and the northeastern lowlands (MARKOV 1992a). For comparison with Central European

populations of the three species, samples from France, Germany, Austria, Slovakia, and Slovenia were screened additionally. Thereby it was attempted to obtain specimens from at least two species per sampling area.

Material and methods

Liver, kidney and heart of 75 red deer, 121 roe deer and 155 wild boars from various sampling sites (3 in the red deer, 5 in the roe deer, and 8 in the wild boar) in Bulgaria (Fig. 1) were collected during two hunting seasons (1990, 1991). Although being available in different numbers, in all species the samples cover populations from both field and forest/mountain biotopes. The habitat of sampling sites R3, r4, W1, W2, W3 and W8 consists of continuous mountaneous forests, that of R1, R2, r1, r2, W4, W6, W7 of large agricultural areas with small forest patches, and that of r3, r5, and W5 represents a transition between both extremes (Fig. 1).

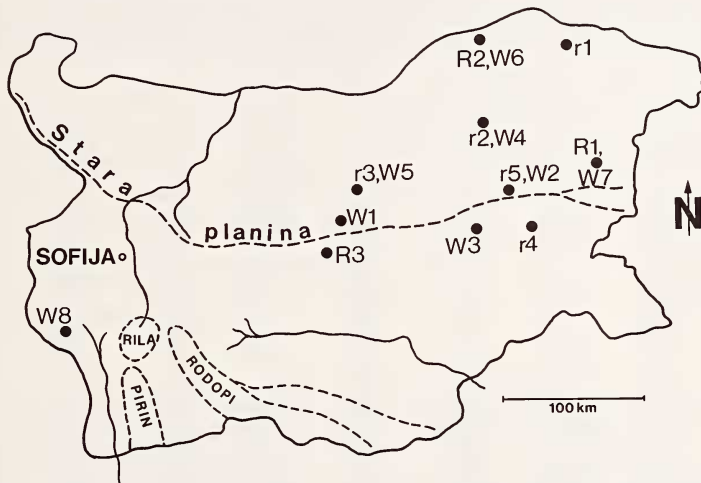


Fig. 1. Distribution of sampling sites of Bulgarian red deer (R1–R3), roe deer (r1–r5), and wild boars (W1–W8). Samples from field habitats (areas with extensive agriculture—: R1, R2, r1, r2, W4, W6, W7; from montaneous forest habitats: R3, r4, W1, W2, W3, W8; from transitional habitats: r3, r5, W5. Dashed lines: main crest of the Balkan (Stara planina), and mountains in southwestern Bulgaria

In addition, samples from the following populations of other parts of Europe were collected: Grosuplje (Gr – SLO, 19 roe deer), Sitno, Polana (Si, Po – ČSFR, 16 roe and 57 red deer), Eisenstadt (Ei – enclosure in southeastern Austria – comp. HARTL and CSAIKL 1987; HARTL 1991, 27 wild boars, culled in 1990), Achenkirch (Ak – western Austria, 35 roe deer, 46 red deer) Eberbach (Eb – near Heidelberg, Germany, 16 wild boars), and the Northern Vosges (NV – France, 27 wild boars, 36 roe deer, data on red deer were taken from HARTL et al. 1991a).

Preparation of tissue extracts, horizontal starch gel electrophoresis and enzyme specific staining procedures were performed as described previously (HARTL and HÖGER 1986; GRILLITSCH et al. 1992). The following 33 isozyme systems were screened (abbreviation, E.C. number and tissues are given in parentheses – L = liver, K = kidney, H = heart): α -glycerophosphate dehydrogenase (GDC, E.C. 1.1.1.8, L), sorbitol dehydrogenase (SDH, E.C. 1.1.1.14, L), lactate dehydrogenase (LDH, E.C. 1.1.1.27, K), malate dehydrogenase (MDH, E.C. 1.1.1.37, K), malic enzyme (ME, E.C. 1.1.1.40, K), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42, K), 6-phosphogluconate dehydrogenase (PGD, E.C. 1.1.1.44, K), glucose dehydrogenase (GDH, E.C. 1.1.1.47, L), glucose-6-phosphate dehydrogenase (GPD, E.C. 1.1.1.49, K), xanthine dehydrogenase (XDH, E.C. 1.2.3.2, L), glutamate dehydrogenase (GLUD, E.C. 1.4.1.3, L), NADH-diaphorase (DIA, E.C. 1.6.2.2, L, K), catalase (CAT, E.C. 1.11.1.6, K), superoxide dismutase (SOD, E.C. 1.15.1.1, K), purine nucleoside phosphorylase (NP, E.C. 2.4.2.1, K), aspartate aminotransferase (AAT, E.C. 2.6.1.1, K), hexokinase (HK, E.C. 2.7.1.1, K, H), pyruvate kinase (PK, E.C. 2.7.1.40, H), creatine kinase (CK, E.C. 2.7.3.2, K, H), adenylate kinase (AK, E.C. 2.7.4.3, K, H), phosphoglucomutase (PGM, E.C.

2.7.5.1, K), esterases (ES, E.C. 3.1.1.1, K), acid phosphatase (ACP, E.C. 3.1.3.2, K), fructose-1,6-diphosphatase (FDP, E.C. 3.1.3.11, K), β -galactosidase (β -GAL, E.C. 3.2.1.23, L), peptidases (PEP, E.C. 3.4.11, K), aminoacylase-1 (ACY-1, E.C. 3.5.1.14, K), adenosine deaminase (ADA, E.C. 3.5.4.4, L, K), aldolase (ALDO, E.C. 4.1.2.13, H), fumarate hydratase (FH, E.C. 4.2.1.2, L), aconitase (ACO, E.C. 4.2.1.3, K), mannosephosphate isomerase (MPI, E.C. 5.3.1.8, K), and glucosephosphate isomerase (GPI, E.C. 5.3.1.9, K).

The interpretation of electrophoretic band-patterns followed the principles outlined by HARRIS and HOPKINSON (1976) and HARRIS (1980). Alleles were designated according to the relative electrophoretic mobility of the corresponding allozymes. In wild boars, at each polymorphic locus the most common allozyme in the Austrian population was designated "100". In the red deer and the roe deer, most of the alleles were already defined in HARTL and REIMOSER (1988) and HARTL et al. (1990a, 1991a, b). The proportion of polymorphic loci (P, 99 per cent criterion), expected (H) and observed (H_o) average heterozygosity were calculated according to AYALA (1982). Genetic distances were calculated according to NEI (1972, 1978). Dendrograms were constructed by various methods reviewed in HARTL et al. (1990b) using the PHYLIP-programme package of FELSENSTEIN (1985), the stability of clusters was examined by means of the jackknife and bootstrap method as described in the same paper. In cases where unique alleles occurred in one or the other sample, the theoretical amount of gene flow among populations was estimated using SLATKIN's (1985) concept of 'private alleles' [$\bar{p}(i)$]. Since only few private alleles occurred, the conditional average frequency [$\bar{p}(i)$] for all alleles

Table 1. Allele frequencies and genetic variation in red deer

Locus	Allele	R1 (28)	R2 (13)	R3 (34)	Po (28)	Si (29)	Ak (46)	NV (233)
Me-1	100	0.714	0.833	0.647	0.700	0.938	0.851	0.983
	125	0.286	0.167	0.353	0.280	0.031	0.096	0.004
	90	0.0	0.0	0.0	0.020	0.031	0.053	0.013
Me-2	100	0.413	0.458	0.371	0.440*	0.625	0.707	0.635
	110	0.587	0.542	0.629	0.560	0.375	0.293	0.365
Idh-2	100	0.768	0.792	0.794	0.518*	0.655	0.415	0.423
	125	0.232	0.208	0.206	0.482	0.345	0.585	0.543
	112	0.0	0.0	0.0	0.0	0.0	0.0	0.034
Sod-2	-100	0.821	0.955	0.853	0.929	0.914	0.925	0.983
	-200	0.179	0.045	0.147	0.071	0.086	0.075	0.017
Pgm-2	100	0.740	0.875	0.677	0.964	0.983	0.957	0.974
	94	0.056	0.0	0.0	0.0	0.0	0.0	0.0
	89	0.148	0.125	0.226	0.036	0.017	0.011	0.026
	79	0.056	0.0	0.097	0.0	0.0	0.032	0.0
Acp-1	100	0.685*	0.542	0.885	0.607*	0.603	0.223	0.773
	300	0.315	0.458	0.115	0.393	0.397	0.777	0.227
Acp-2	100	0.423	0.292	0.307	0.185	0.034	0.478*	0.452
	85	0.269	0.416	0.597	0.815	0.966	0.522	0.548
	73	0.308	0.292	0.096	0.0	0.0	0.0	0.0
Mpi	100	0.964	0.962	0.868	1.0	1.0	0.862	1.0
	132	0.018	0.038	0.014	0.0	0.0	0.138	0.0
	70	0.018	0.0	0.118	0.0	0.0	0.0	0.0
Gpi-1	-100	0.982	0.923	0.971	1.0	1.0	1.0	1.0
	60	0.018	0.077	0.029	0.0	0.0	0.0	0.0
	P	0.209	0.209	0.209	0.163	0.163	0.186	0.163
	H	0.073	0.065	0.070	0.055	0.040	0.066	0.045
	H_o	0.073	0.066	0.072	0.037	0.036	0.065	0.043

R1-R3 = Bulgaria, Po = Polana (ČSFR), Si = Sitno (ČSFR), Ak = Achenkirch (A), NV = Northern Vosges (F). Sample sizes are given in parentheses. P = proportion of polymorphic loci (99 % criterion), H (H_o) = expected (observed) average heterozygosity. P, H, and H_o are calculated over 43 loci.

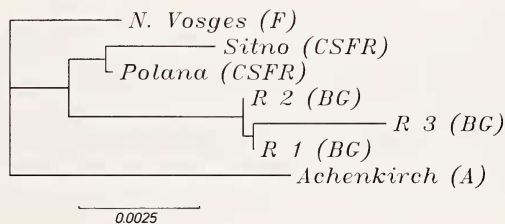
* observed genotypes deviated significantly from Hardy-Weinberg equilibrium.

was plotted against i/d , where i is the number of samples containing a particular allele and d is the total number of samples studied (SLATKIN 1981). This method does not permit a direct estimation of N_m , but gives an overall impression of the distribution of alleles among populations in relation to their frequencies. In the case of undisturbed migration, the number of samples in which an allele is present should monotonously increase together with an increase of the average frequency of the respective allele. Furthermore, G -statistics (NEI 1975) were used to assess relative differentiation among populations and also to estimate N_m from F_{ST} (in a broader sense) as outlined by SLATKIN and BARTON (1989).

Results

In order to obtain data fully comparable to those reported in previous studies (comp. HARTL et al. 1991a, b), not all isozymes were investigated in each species. In the red deer 43 presumptive structural loci were scored, 9 of which were polymorphic: Me-1, Me-2, Idh-2, Sod-2, Pgm-2, Acp-1, Acp-2, Mpi, and Gpi-1. Whereas all these loci were already found polymorphic by HARTL et al. (1990a, 1991a), some novel alleles were detected: Pgm-2⁹⁴, Acp-2⁷³, and Mpi⁷⁰. Allele frequencies in the various samples and indices of genetic variation are given in table 1. With the remarkable exception of Polana, where a significant deficiency of heterozygotes was detected at three loci (Me-2, Idh-2, Acp-1), in the other samples genotypes at (almost) all polymorphic loci were in Hardy-Weinberg equilibrium. Calculated over the Bulgarian samples, mean $P = 0.209$ (sd. 0), mean $H = 0.069$ (sd. 0.004), $G_{ST} = 0.038$, N_m (calculated from $G_{ST} - I$) = 6.33, N_m (calculated from $\bar{p}(1 - I)$) = 2.40, and mean NEI's (1978) $D = 0.0023$ (sd. 0.0021). Calculated over all samples, mean $P = 0.186$ (sd. 0.023), mean $H = 0.059$ (sd. 0.013), $G_{ST} = 0.096$, $N_m(I) = 2.35$, $N_m(II) = 1.58$, and mean $D = 0.0076$ (sd. 0.0044). Genetic relationships among populations are shown in figure 2.

Fig. 2. Unrooted dendrogram, showing genetic relationships in red deer (NEI's 1978 D/FITCH-MARGOLIASH tree). A rooted dendrogram (NEI's 1978 D/UPG-MA) was topologically identical and stable both with respect to sample sizes (bootstrap) and the composition of loci chosen (jackknife)



In the roe deer 8 out of 40 loci were polymorphic: Dia-2, Ak-1, Pgm-1, Pgm-2, Acp-1, Pep-2, Mpi, and Gpi-1. All loci were previously found polymorphic in this species (HARTL and REIMOSER 1988; HARTL et al. 1991b), but a novel allele, Pep-2¹²⁰, occurred exclusively in Bulgaria and Slovenia. Allele frequencies and indices of genetic variation are given in table 2. Calculated over the Bulgarian samples, mean $P = 0.175$ (sd. 0), mean $H = 0.065$ (sd. 0.003), $G_{ST} = 0.044$, $N_m(I) = 5.43$, and mean $D = 0.0006$ (sd. 0.0005). Calculated over all samples, mean $P = 0.178$ (sd. 0.008), mean $H = 0.059$ (sd. 0.010), $G_{ST} = 0.064$, $N_m(I) = 3.69$, $N_m(II) = 37.38$, and mean $D = 0.0025$ (sd. 0.0021). Genetic relationships among populations are shown in figure 3.

In wild boars 40 loci were examined. Nine of them were polymorphic: Me-1, Mdh-2, Dia, Pgm-2, Pgm-3, Pep-1, Acy-1, Ada-2, Gpi-1 (some of these polymorphisms were already described in Austrian wild boars by HARTL and CSAKL 1987). Allele frequencies and indices of genetic variation are given in table 3. The following 31 loci were monomorphic: Gdc, Sdh, Ldh-1, -2, Mdh-1, Me-2, Idh-1, -2, Pgd, Gpd, Xdh, Glud, Cat, Sod-1, -2, Np, Aat-1, -2, Hk, Ck-2, Pgm-1, Acp-1, -2, Fdp, β -Gal, Pep-2, Fh, Aco-1, -2, Mpi,

Table 2. Allele frequencies and genetic variation in roe deer

Locus	Allele	Sample									
		r1 (33)	r2 (21)	r3 (27)	r4 (23)	r5 (17)	Gr (19)	Si (16)	Ak (35)	NV (36)	
Dia-2	100	0.939	0.976	0.963	0.913	0.941	0.842	0.969	0.986	0.972	
	118	0.061	0.024	0.037	0.087	0.059	0.158	0.031	0.014	0.028	
Ak-1	100	0.258*	0.309*	0.315	0.130	0.294	0.237	0.313	0.143	0.167	
	250	0.742	0.691	0.685	0.870	0.706	0.763	0.687	0.857	0.833	
Pgm-1	100	0.818	0.810	0.796	0.804	0.853	0.947	0.969	0.886	0.986	
	-16	0.182	0.190	0.204	0.196	0.147	0.053	0.031	0.114	0.014	
Pgm-2	100	0.750	0.790*	0.717*	0.658	0.546	0.605	0.500	0.714	0.615	
	113	0.025	0.026	0.087	0.105	0.272	0.0	0.031	0.200	0.071	
	70	0.225	0.184	0.196	0.237	0.182	0.395	0.469	0.086	0.314	
Acp-1	100	0.685	0.474	0.546	0.650	0.567*	0.658	0.750	0.912	0.871	
	200	0.315	0.526	0.454	0.350	0.433	0.342	0.250	0.088	0.129	
Pep-2	100	0.267	0.306	0.282	0.238*	0.235	0.286	0.400	0.400	0.560	
	120	0.167	0.027	0.109	0.0	0.118	0.107	0.0	0.0	0.0	
	115	0.483	0.417	0.370	0.452	0.500	0.321	0.433	0.557	0.440	
	107	0.083	0.250	0.239	0.310	0.147	0.286	0.167	0.043	0.0	
Mpi	100	0.879	0.738	0.889	0.717	0.853	0.947	0.875	0.900	0.972	
	120	0.121	0.262	0.111	0.283	0.147	0.053	0.125	0.100	0.028	
Gpi	100	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.986	1.0	
	300	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.014	0.0	
	P	0.175	0.175	0.175	0.175	0.175	0.175	0.175	0.200	0.175	
	H	0.062	0.067	0.064	0.065	0.069	0.062	0.057	0.045	0.041	
	H _o	0.063	0.065	0.065	0.058	0.057	0.059	0.056	0.045	0.042	

r1-r5 = Bulgaria, Gr = Grosuplje (SL), Si = Sitno (ČSFR), Ak = Achenkirch (A), NV = Northern Vosges (F). Sample sizes are given in parentheses. P = proportion of polymorphic loci (99 % criterion), H (H_o) = expected (observed) average heterozygosity. P, H, and H_o are calculated over 40 loci.
* observed genotypes deviated significantly from Hardy-Weinberg equilibrium.

and Gpi-2. Calculated over the Bulgarian samples, mean P = 0.122 (sd. 0.028), mean H = 0.029 (sd. 0.006), G_{ST} = 0.094, Nm(I) = 2.41, and mean D = 0.0062 (sd. 0.0217). Calculated over all samples, mean P = 0.116 (sd. 0.028), mean H = 0.028 (sd. 0.005), G_{ST} = 0.125, Nm(I) = 1.75, Nm(II) = 27.73, and mean D = 0.0032 (sd. 0.0029). Genetic relationships among populations are shown in figures 4 and 5.

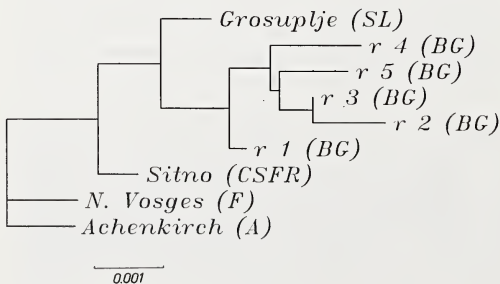


Fig. 3. Unrooted dendrogram, showing genetic relationships in roe deer (FITCH-MARGOLIASH tree, since negative distances are not allowed NEI's 1972 D was used). A rooted dendrogram, (NEI's 1978 D/UPGMA) was topologically identical and stable both with respect to sample sizes (bootstrap) and the composition of loci chosen (jackknife)

Fig. 4. Unrooted dendrogram, showing genetic relationships in wild boars (FITCH-MARGOLIASH tree, since negative distances are not allowed NEI's 1972 D was used)

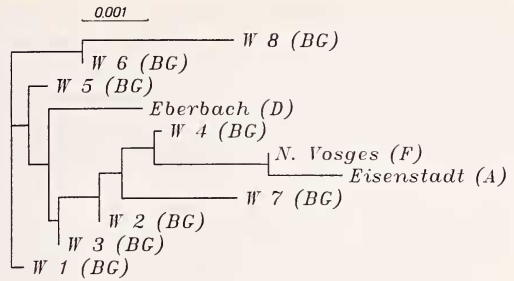
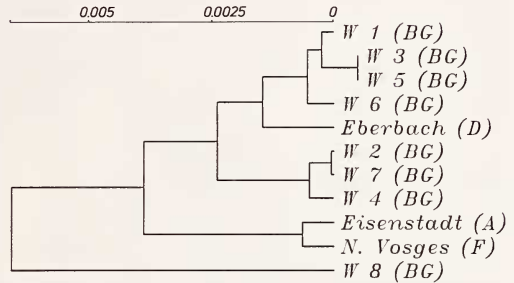


Fig. 5. Rooted dendrogram, showing genetic relationships in wild boars (NEI's 1978 D/UPGMA). The dendrogram was topologically unstable with respect to sample sizes (bootstrap), but stable with respect to the composition of loci chosen (jackknife)



Discussion

Red deer

Both with respect to the proportion of polymorphic loci and average heterozygosity, biochemical-genetic variation in the Bulgarian red deer is higher than in any other European population examined so far (comp. HARTL et al. 1990a, 1991a). Since novel alleles were detected and relative as well as absolute genetic differentiation were considerably smaller among only Bulgarian than among all samples, our data suggest that the red deer in that area is different from Central European *Cervus elaphus bippelaphus*. Genetic distances (Fig. 2) from French (NV), Austrian (Ak) or Czechoslovakian (Si) populations are of a magnitude described previously for other European subspecies of *Cervus elaphus* by GYLLENSTEN et al. (1983).

Red deer in Slovakia was extinct at the beginning of the 19th century and restocked in the Polana area – among others – with animals representing *C. e. carpathicus*. In our data, there is a deficiency of heterozygotes at several loci, possibly indicating a Wahlund effect (Tab. 1). Since there appear to be no geographical barriers within this area, the latter may be due to the formation of local demes as it has been discussed by SCHREIBER et al. (1992). Also the Austrian population (Ak) may contain not only autochthonous red deer, which is indicated by the comparatively large distance from all other populations studied (Fig. 2) and the presence of an MPI-polymorphism, lacking in all pure populations of Central European red deer examined so far (comp. Tab. 1, and GYLLENSTEN et al. 1983; HARTL et al. 1990a, 1991a), but being present in *C. e. scoticus* (PEMBERTON et al. 1988) and *C. e. canadensis* (DRATCH and GYLLENSTEN 1985). Attempts to introduce animals from the latter subspecies in Bavaria have been reported by BENINDE (1937).

Roe deer

Genetic variability in Bulgarian, Slovenian and Slovakian roe deer is high when compared to that of populations of Austria, Switzerland, France and Hungary (Tab. 2, and HARTL et

al. 1991b). This is due to the ubiquitous occurrence of many variant alleles showing only a scattered distribution in the populations examined by HARTL *et al.* (1991b) rather than to the presence of new polymorphisms. This result corresponds to the extremely low D - and G_{ST} -values within Bulgaria, suggesting the absence of any factors disturbing gene flow within the study area. Indeed, N_m within Bulgaria is the highest yet detected in this species (see HARTL *et al.* 1991b). The considerable difference between N_m as estimated from G_{ST} or from $\bar{p}(1)$ in the present study may be the result of sampling bias in the latter approach – only one private allele occurred). The close relationship among Bulgarian roe deer populations is in accordance with craniometric, somatometric and cytogenetic data (MARKOV and DOBRIJANOV 1985; MARKOV *et al.* 1991a, b).

As an adaptation to increasing deforestation, in the roe deer the existence of a distinct 'field ecotype' has been postulated by PIELOWSKI (1970), which differs from the classical 'forest ecotype' in various non-metric morphological, biochemical, physiological, and behavioural characteristics (e.g. PIELOWSKI 1977; MAJEWSKA *et al.* 1982; MARKOWSKI and MARKOWSKA 1988; KURT 1991). In our data, the pure representatives of both 'ecotypes' (r_1 , r_4) did not show a genetic distance higher than typical for local populations (Fig. 3). This suggests that the 'field ecotype' reflects the adaptive potential of the species rather than a particular genetic integrity, whereby roe deer living in transitional habitats obviously play an important role in maintaining the gene flow between both extreme types (Figs. 1, 3).

Except for the Hungarian roe deer (HARTL *et al.* 1991b), overall genetic distances among the populations studied so far do not suggest the presence of different subspecies in Europe. HARTL *et al.* (1991b) proposed that the large average genetic distance ($D = 0.0112$) of the Hungarian roe deer from Austrian and Swiss populations may be the result of the completely fenced boarder existing between Austria and both Hungary and Czechoslovakia from the late 1940s to the late 1980s. Since the D -value between the Slovakian and the Austrian or the French roe deer is quite small (Fig. 3) this hypothesis is not supported by the present study.

Wild boars

In wild boars, genetic variation in the Bulgarian samples is not remarkably different from that in the other areas studied (Tab. 3). RANDI *et al.* (1992) found similar values of average heterozygosity, but a lower proportion of loci polymorphic which is probably due to a partially different set of proteins examined by these authors. In contrast to the roe and the red deer, the Bulgarian samples do not form a separate cluster (Figs. 4, 5), but are interspersed with Central European populations and, according to the bootstrap, all the clusters shown in figure 5 are not very stable. The Austrian population (Ei) is living in an enclosure, which was monitored by our laboratory from 1984 to 1990 by screening samples of an average size of 33 (sd. 9) specimens for allozyme polymorphism at the set of loci given in the present study every year. The population of about 300 individuals is annually reduced to one half both by battues and by selective elimination of 'weak' yearlings (for details see HARTL 1991). Average N_{E1} 's (1978) pairwise D among samples from seven consecutive years with 0.0039 (sd. 0.0031) is of the same magnitude as that among samples from the different locations examined in the present study (0.0032, sd. 0.0029). Although temporal changes of allele frequencies may be less extensive in free-ranging populations, this example shows that, probably due to the smaller number of polymorphic loci and the much smaller number of rare alleles, the pattern of geographical differentiation in wild boars may be less reliable than in the red or the roe deer. Nevertheless, population W8 is well separated from all other wild boars in the dendrogram (Fig. 4), which is in accordance with results based on 38 skull measurements (MARKOV 1992b). These data support the hypothesis, that the animals in the area of the Rila-Rodopi mountain massiv belong to an other subspecies than those from the eastern part of the

Table 3. Allele frequencies and genetic variation in wild boars

Locus	Allele	Sample										
		W1 (30)	W2 (15)	W3 (40)	W4 (25)	W5 (12)	W6 (15)	W7 (9)	W8 (9)	Ei (27)	Eb (16)	NV (27)
Me-1	100	0.966	1.0	1.0	0.905	0.950	1.0	0.889	1.0	0.982	1.0	0.907
	117	0.034	0.0	0.0	0.095	0.050	0.0	0.111	0.0	0.018	0.0	0.093
Mdh-2	100	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.982
	136	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.018
Dia	100	0.950	0.821	0.800	0.938	0.792	0.800	0.778	0.889	1.0	0.700	1.0
	132	0.050	0.179	0.200	0.062	0.208	0.200	0.222	0.111	0.0	0.300	0.0
Pgm-2	100	0.536	0.750	0.618	0.796	0.500	0.385	0.875	0.188	0.706	0.625	0.783
	113	0.464	0.250	0.382	0.204	0.500	0.615	0.125	0.812	0.294	0.375	0.217
Pgm-3	100	1.0	1.0	0.988	0.978	0.958	1.0	1.0	1.0	1.0	1.0	1.0
	110	0.0	0.0	0.012	0.022	0.042	0.0	0.0	0.0	0.0	0.0	0.0
Pep-1	100	0.933	0.900	0.938	0.980	0.875	0.933	1.0	1.0	0.667	1.0	0.769
	92	0.067	0.100	0.062	0.020	0.125	0.067	0.0	0.0	0.333	0.0	0.231
Acy-1	100	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.982
	123	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.018
Ada-2	100	0.867*	0.800	0.800*	0.875	0.750	0.923	0.667	0.778	1.0	1.0	1.0
	150	0.133	0.200	0.200	0.125	0.250	0.077	0.333	0.222	0.0	0.0	0.0
Gpi-1	-100	1.0	1.0	0.988	1.0	1.0	1.0	0.944	1.0	0.852	0.906	1.0
	-45	0.0	0.0	0.012	0.0	0.0	0.0	0.056	0.0	0.148	0.094	0.0
	P	0.125	0.100	0.150	0.150	0.150	0.100	0.125	0.075	0.100	0.075	0.125
	H	0.025	0.029	0.032	0.023	0.040	0.027	0.033	0.021	0.029	0.027	0.023
	H _o	0.025	0.022	0.033	0.018	0.042	0.027	0.037	0.014	0.026	0.025	0.023

W1-W8 = Bulgaria, Ei = Eisenstadt (A), Eb = Eberbach (D), NV = Northern Vosges (F). Sample sizes are given in parentheses. P = proportion of polymorphic loci (99 % criterion), H (H_o) = expected (observed) average heterozygosity. P, H, and H_o are calculated over 40 loci.
* observed genotypes deviated significantly from Hardy-Weinberg equilibrium.

country (MARKOV 1954). Considering the different distribution of sampling sites in both studies, our results are more or less in accordance with those of RANDI et al. (1992). Like in the roe deer, the difference between Nm as estimated from G_{ST} or from $\bar{p}(1)$ may be the result of sampling bias in the latter (only two private alleles occurred).

Comparative aspects

In contrast to previous studies (HARTL and REIMOSER 1988; HARTL et al. 1991b), mean P- and H-values were very similar between the red and the roe deer, which is probably the result of collecting samples from the same sites or at least from similar habitats, respectively. The number of populations in which an allele is present increases monotonously with its average frequency in all three species, which, together with the similar Nm (when calculated from G_{ST}), indicates a similar amount of migration in all three species. Only in the red deer there is an exception as to alleles occurring in four samples (Me-1⁹⁰, Mpi¹³² - Tab. 1), where the expected average frequency is much too low. This could possibly be the result of natural (Mpi¹³², PEMBERTON et al. 1988) or artificial (Me-1⁹⁰, HARTL et al. 1991a) selection discriminating against these alleles.

In the roe and the red deer, the Bulgarian samples are well separated from the Central European populations and (as far as samples are available) are more closely related to those from Slovenia and Slovakia than to those from Austria or France. According to the

exclusive occurrence of rare alleles in both species (Tab. 1, 2) Bulgarian roe and red deer represent a distinct gene pool which should neither be contaminated by introductions of deer from abroad nor serve as a source for restocking in Central Europe. At least with regard to the population living in the Rila/Rodopi mountains this statement is valid also for wild boars.

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Zusammenfassung

Allozymdiversität innerhalb und zwischen Populationen dreier Ungulatenarten (Cervus elaphus, Capreolus capreolus, Sus scrofa) aus Südost- und Mitteleuropa

Mittels horizontaler Stärkegelelektrophorese wurde bei 75 Rothirschen, 121 Rehen und 155 Wildschweinen aus verschiedenen Regionen Bulgariens die genetische Variabilität und Differenzierung an 40–43 Genloci untersucht. Zum Vergleich mit mitteleuropäischen Populationen wurden weitere 103 Rothirsche, 106 Rehe und 70 Wildschweine aus Probengebieten in Slowenien, Österreich, der Slowakei und Frankreich herangezogen. Der durchschnittliche Polymorphiegrad (P, 99 % Kriterium) betrug beim Rothirsch 18,6 % (sd. 2,3 %) und beim Reh 17,8 % (sd. 0,8 %), die durchschnittliche Heterozygotierate betrug bei beiden Arten 5,9 % (sd. 1,3 % bzw. 1,0 %). Sowohl beim Rothirsch als auch beim Reh waren der Polymorphiegrad und die Heterozygotierate der bulgarischen, slowenischen und slowakischen Populationen höher als die meisten bisher beschriebenen P- und H-Werte. Die genetischen Distanzen zu anderen Beständen und das ausschließliche Vorkommen bestimmter Allele unterscheiden die bulgarischen Rothirschpopulationen auf dem (elektrophoretischen) Niveau einer Unterart vom mitteleuropäischen *Cervus elaphus hippelaphus*. Die Wildschweine waren im Durchschnitt an 11,6 % (sd. 2,8 %) der Loci polymorph und zeigten einen mittleren Heterozygotiegrad von 2,8 % (sd. 0,5 %). Nach den genetischen Distanzen unterscheidet sich die Population in den Rila-Rodopi-Bergen deutlich von der mitteleuropäischen Unterart *Sus scrofa scrofa*. Der Migrationsgrad (Nm) zwischen den Populationen war bei allen drei Arten sehr ähnlich.

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